FABRICATION OF BIO COMPOSITE FROM
BACTERIAL CELLULOSE/STARCH

SITI NATRAH BINTI ISMAIL

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ABSTRACT

Bio composite from starch and bacterial cellulose offer good mechanical properties. The objective of this study was to fabricate bio composite from starch, and bacterial cellulose. The research was conducted with 25 samples with difference composition of bacterial cellulose (BC) (0, 7, 14, 21 and 28) wt% and starch (3, 6, 9, 12 and 15) wt%. The bacterial cellulose film produced by *Acetobacter xylinum* was blend and mixed with starch, and glycerol. The film was characterized by using Universal Testing Machine, water absorption, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Soil Burial Degradation, biodegradation by using *Aspergillus niger*. When the composition of starch and BC increased, the tensile strength and Tensile modulus was increased and the elongation at break was decreased. The absorption of water was increased proportionate with the increasing the composition of bacterial cellulose and starch. From the FTIR test, the bio composite was showed the present of hydroxyl group, C=O stretching (amide I), and C-O bonding. Besides that, TGA showed the thermal degradation of starch and cellulose occurs when the temperature arise to 280°C, leads to depolymerization and to the formation of 1,6-anhydroglucose-ß-D-glucopyranose. Moreover, the SEM analysis showed a smooth and homogenous structure of the film but when the composition of BC increased, the small mat fragments can be saw and the layered structure becomes clearer. The soil burial test and degradation by using *Aspergillus niger* indicated the degradation rate decreased as the bacterial cellulose content increased. As a conclusion, the film fabricated had a potential application in future to be used as food packaging material because as it had good mechanical properties and biodegradable. In order to improve the properties of bio composite, more research should be done to know the optimum composition of BC and starch should be used to produce the film. Besides that, the study about other alternative material or additive that can be added to the film also can be done to increase the mechanical properties and degradation time.

ABSTRAK

Kanji mempunyai struktur yang lemah sebagai termoplastik, untuk meningkatkan sifat mekanik bio komposit, kajian telah dilakukan dengan menambah dan selulosa bakteria di dalam bio komposit kanji. Tujuan kajian ini adalah untuk menghasilkan bio komposit dari kanji dan Selulosa Bakteria. Penyelidikan dilakukan dengan menggunakan 25 sampel dengan perbezaan komposisi selulosa bakteria (0, 7, 14, 21 dan 28) wt% dan kanji (3, 6, 9, 12 and 15) wt%. Filem selulosa bakteria yang dihasilkan oleh *Acetobacter xylinum* dikisar dan dicampur dengan kanji dan glycerin. Filem tersebut akan dikategorikan dengan menggunakan Universal mesin Testing, kadar penyerapan air, Fourier Transform Infrared (FTIR) Spektroskopi, Thermo Gravimetric Analysis (TGA) dan Mikroskop Elektron (SEM) ujian degradasi tanah, dan ujian degradasi dengan menggunakan *Aspergillus niger*. Apabila komposisi kanji dan BC meningkat, kekuatan tegangan dan kekenyalan tegangan akan meningkat dan pemanjangan pada waktu rehat akan berkurangan. Kadar penyerapan air telah meningkat berkadar dengan peningkatan kandungan selulosa bakteria dan kanji. Daripada ujian FTIR, biocomposite menunjukkan yang hadir kumpulan amide I, ikatan C-O dan ikatan OH. Selain itu, TGA menunjukkan degradasi haba daripada selulosa berlaku apabila suhu meningkat pada 280°C, membawa kepada depolymerization dan pembentukan 1,6-anhydroglucose-ß-D-glucopyranose. Ujian degradasi tanah dan degradasi dengan menggunakan *Aspergillus niger* menunjukkan penurunan kadar penyingkiran apabila kadar selulosa bakteria meningkat. Kesimpulannya, filem telah dibuat berpotensi di masa depan untuk digunakan sebagai pembungkus makanan kerana mempunyai sifat mekanik yang baik, dan terbiodegradasikan. Untuk meningkatkan ciri-ciri bio komposit, lebih banyak kajian perlu dilakukan untuk mengetahui komposisi selulosa bacteria dan kanji yang perlu digunakan untuk menghasilkan filem. Selain itu, kajian mengenai bahan gantian atau tambahan yang boleh di tambah ke dalam filem boleh dilakukan untuk menambah ciri-ciri mekanikal dan masa untuk mengurai.

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LIST OF SYMBOLS

^oC	Celsius
$^oC/min$	Celsius per minutes
cm	Centimeter
cm^{-1}	Per centimeter
cm^2/min	Centimeter square per min
g	Gram
h	Thickness of the film
k	Slope
kN	Kilo Newton
min	Minutes
mL min^{-1}	Milliliter per minutes
mm	Millimeter
MPa	Mega Pascal
Mt	Mass gain in time
$M\infty$	Mass gain at equilibrium (maximum water uptake)
nm	Nanometer
W_o	Initial Mass
W_t	Remaining Mass
w/v	Weight per volume
w/w	Weight per weight
µm	Micrometer

α	Alpha – glycoside link
β	Beta – glycoside link
ε	Elongation at break
%	Percent

LIST OF ABBREVIATIONS

BC	Bacterial cellulose
BCC	Bamboo Cellulosic Crystals
D	Diffusivity
DI	Deionize
FTIR	Fourier Transform Infrared Spectroscopy
IR	Infrared
PS	Plastic Starch
SEM	Scanning Electron Microscope
TC	Terminal Complex
TGA	Thermo Gravimetric Analysis
TPS	Thermoplastic Starch
TM	Tensile Modulus
TS	Tensile Strength

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Petrochemical polymers commonly called as “plastics” have been booming and are by far the most widely used polymers for packaging. Plastic have become very popular with consumers in developing countries, as there are cheap, strong, lightweight, and functional. One of the useful characteristics of plastic is the fact that it is durable. Unfortunately, this is not a positive characteristic when it comes to the environment. The fact that plastic is a durable meaning it cannot be degraded. Over-use of plastic bags extremely causes pollution and damage to the environment. Plastic waste is recognized as one of the most troublesome categories of waste, and disposal of plastic waste has been blamed for shortening the life of landfill sites (Ishigaki et al., 2004). In addition, burning plastic can sometimes result in toxic fumes and risks to the health of the planet.

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The increasing of a waste problem from the usage of petrochemical polymers can be overcome with the use of bio composite. The material component such as natural fiber and biodegradable polymer has been used as the alternative materials in producing the new bio composites. It is safe to be used in food industry as the packaging material and in fact free from toxic. The challenge in producing food packaging is to match the durability of the packaging with the product shelf-life. Liu (2006) list up the factors that can degrade food quality which are the environmental temperature, relative humidity, presence of active bacterial and spoilage microorganisms and ultraviolet exposure. These factors also can influence the rate of degradation of the bio composite material.

There are several of bio-based materials which can be used as innovative applications in food-related packaging. These materials include starch, cellulose, and those derived from microbial fermentation. From all these material, Fama et al., (2009) state that starch is potential to be material for biodegradable plastics because it can form a continuous matrix besides as a renewable and abundant resource. Starch is the major carbohydrate in plant tubes and seed endosperm, where it is found as granules. Each granule contains amylopectin molecules together with a larger number of smaller amylose molecules (Canigueral et al., 2009). Corn, wheat, beets, sugar, potatoes and other plants, as well as vegetable oils are the main materials from which the bio composite is produced. According to Martins et al., (2009), starch can be converted into a thermoplastic material, known as thermoplastic starch (TPS), through the disruption of the molecular chain interactions under specific conditions, in the presence of a plasticizer.

Cellulose is the most abundant biopolymer on earth, recognized as the major component of plant biomass, but also as a representative of microbial extracellular polymers. Such as bacterial cellulose (BC) is synthesized by the *Acetobactor xylinum* bacteria. BC is been used widely in the production of bio composite (Shoda and Sugano, 2005). It is because it has remarkable mechanical properties in both wet and dry states, porosity, water absorbency, moldability, biodegradability and excellent biological affinity.

1.2 PROBLEM STATEMENT

Nowadays, the largest part of all materials used in the packaging industries is produced from fossil fuels and practically non-biodegradable. Plastics have been an environmental trepidation because of the non-degrade behaviour. The chemical constituents of plastic wastes can break down and release toxins that harm the environment, animals and the human being. The amount of plastic waste increases every year, but the exact time needed for its degradation is unknown. Chan-Halbrendt et al. (2009) analyzed the data around the world and conclude that around 500 billion plastic bags were used worldwide every year. Normally, a single plastic can take up to 1000 years, to decay completely. The world is also running out of landfill space as degradation of plastics requires a long period of time and most of them end up to overburdening the landfill (Xu et al., 2005).

Bio composite is a suitable material that can substitute the usage of conventional plastic. However, the selection of raw materials and low cost and hold quality production is important in bio composite. Hence, this research will investigate the production of biodegradable composite from different composition of bacterial cellulose and starch. Starch composite will cause a weak structure of the bio composite. Thus, the additional of bacterial cellulose will improve the tensile strength of the bio composite.

1.3 OBJECTIVE

The objective of this study is to fabricate the bio composite from bacterial cellulose/starch.

1.4 SCOPE

The scopes of the study are:

1) To fabricate bio composite from bacterial cellulose/starch by using casting method by using different composition of bacterial cellulose (0, 7, 14, 21 and 28) wt% and starch (3, 6, 9, 12 and 15) wt%.

2) To characterize the bio composite film by using, Universal Testing machine, water absorption test, Fourier Transform Infrared (FTIR) Spectroscopy, Thermo Gravimetric Analysis (TGA) Scanning Electron Microscopy (SEM), Soil burial degradation test and biodegradation test by using *Aspergillus niger*.

1.5 RATIONAL AND SIGNIFICANCE

Biodegradable plastics are a new generation of polymers newly emerging in the market. The increasing demand for renewable and bio-based materials has shifts the consumer to prefer an eco-friendly packaging and driving the market for global biodegradable plastics. Among of all the market demand, the starch-based plastics have the largest share in bio composite products. It offers tremendous potential for food packaging, and becomes the largest demand due to the increase of consumer awareness for sustainable packaging. This is because it can degrade faster than the conventional plastics and not produce toxic when degrades in the landfills.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Bacterial cellulose and starch is a potential cheap feedstock for bio composite production. In the past, many literatures and papers had been explored to enhance the reliability and cost effective productivity of bio composite using bacterial cellulose and starch. This chapter will review on the production of bio composite and the main composition of composite which are bacterial cellulose (BC) and starch. Besides that, it also reviews on tests that have been used to study the characteristic of bio composite.

2.2 BACTERIAL CELLULOSE

Cellulose is the most abundant biopolymer on earth and recognized as the major component of plant biomass, but also as a representative of microbial extracellular polymers. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1-4) linked D-glucose unit. Cellulose is synthesized by bacteria belonging to the genera of *Acetobacter, Rhizobium, Agrobacterium* and *Sarcina* (Jonas and Farah, 1998).

Bacterial cellulose (BC) is synthesized by several bacterial genera, of which *Acetobacter* strains are best known. An overview of BC producers with its structure of cellulose produced is shown in Table 2.1.

Table 2.1: Type of Genus Cellulose and its structure of bacterial cellulose.

Genus Cellulose	**Structure of bacterial cellulose**
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

Source: Jonas and Farah (1998)

From the research conducted by Retegi et al. (2010), the most efficient producers are a Gram-negative, acetic acid bacterium which is *Acetobacter xylinum*. It has been applied as a model microorganism for basic and applied studies on cellulose and can produce cellulotic bio films. *Acetobacter xylinum* is an obligate aerobe bacterium usually found in vinegar, alcoholic beverages, fruit juices, fruits, and vegetables, and most likely in rotting ones as well (Klemm et al., 2001). BC microfibril have high mechanical properties including tensile strength and modulus high water holding capacity, moldability, crystalline, and biocompatibility (Retegi et al., 2010).

From the properties that have been stated, the production of BC is receiving great attention since they can be used in many fields, including pulp and paper industry (Keshk and Sameshima, 2005). Besides that, Chawla et al. (2009) claims that BC also can be used in various areas including textile industry, food, pharmaceutical, waste treatment, broadcasting, mining and refinery.

2.3 *ACETOBACTER XYLINUM*

Acetobacter xylinum is the most efficient producer of cellulose and has been recently reclassified and included within the novel genus of Gluconacetobacter, as *G. xylinus* together with some other species (*G. hansenii*, *G. europaeus, G. oboediens, and G. intermedius*). *Acetobacter xylinum* is a type of acetic acid bacteria that can synthesize cellulose when grown in a synthetic and complex medium containing glucose. *Acetobacter xylinum* is a rod shaped, aerobic and gram negative bacteria which has an ability to synthesize high quality of cellulose organized as twisting ribbon or microfibrillar bundle (Setyawati et al., 2007).

Acetobacter xylinum can convert various carbon compounds, such as hexoses, glycerol, dihydroxyacetone, pyruvate, and dicarboxylic acids, into cellulose, usually with about 50% efficiency. It will grow at the optimum temperature of 25-30℃ and pH range from 5.6 to 6.2 (Pourremezan et. al., 2009)

Figure 2.1 illustrated the scheme for the formation of bacterial cellulose from *Acetobacter xylinum*. The synthesis of cellulose in *Acetobacter xylinum* occurs between the outer membrane and cytoplasm membrane by a cellulose-synthesizing complex, which is in association with pores at the surface of the bacterium (Jonas and Farah, 1998). According to Klemm et al. (2001), the formation of cellulose begin when the glucan chain aggregates and consist of approximately 6–8 glucan chains are elongated from the terminal complexes. Then, these sub elementary fibrils are assembled to form micro fibrils and lastly it wills form a ribbon.

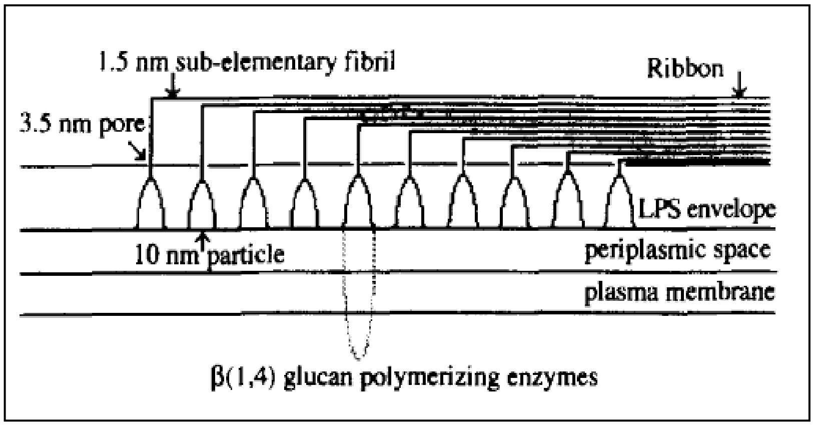

Figure 2.1: Scheme for the Formation of bacterial cellulose from *Acetobacter xylinum.*

Source: Jonas and Farah (1998).

2.4 BIO COMPOSITE

Bio composite is materials that consist of two or more distinct constituent to obtain complex chemical, mechanical and biological properties (Almeida et al., 2010). Biodegradable composites consist of biodegradable polymers as the matrix material and biodegradable fillers. High compatibility occurs between starch matrix and fillers due to the occurrence of intermolecular interactions formed between the different components in the bio composite (Lu et al., 2006). The use of cellulose crystallites as a filler for the preparation of high-performance composite has been explored extensively. When the cellulose crystallites are homogeneously dispersed into polymer matrices, they gave a remarkable reinforcing effect even at a few percent of their concentrations.

From research that conducted by Martins et al. (2009), starch is been used as raw material for development of bio composite. Besides that, incorporating plasticizer agent such as water and glycerol, can make the starch turn into thermoplastic called thermoplastic starch (TPS) or plasticized starch (PS) through destructurization by the introduction of mechanical and heat energy (Carvalho et al., 2003). Owing to hydrophilic attributes of starch, the internal interaction and morphology of starch will be readily changed by water molecules, which allow starch to be successfully injection moulded to obtain TPS (Lu et al., 2009). On the other hand, the hydrophilicity of starch can be used to improve the degradation rate of some degradable hydrophobic polymers. The mixing of natural fiber and TPS will improve the mechanical properties and give good adhesion between reinforcing fibers and the matrix.

2.5 STARCH

Plants store glucose as the polysaccharide starch. Starch is one of the most promising renewable bio resources due to its versatility, competitiveness in price, and applicability to various industries (Liu et al., 2010). The cereal grains (wheat, rice, corn, oats, and barley) as well as tubers such as potatoes are rich in starch. According to Lu et al. (2009), starch is composed of amylose and amylopectin, which are both polysaccharides made up of α–D–glucopyranosyl units linked by (1-4) and (1-6) linkages. The ratio of amylase and amylopectin varies with the starch source, but it is typically 20:80 amylose to amylopectin. Figure 2.2 illustrated the amylase and amylopectin structure. Amylose is a straight chain polymer with an average of 200 glucose units per molecule while amylopectin consist of 1,000 glucose molecule arranged into a branched chain (Dufresne et al., 2000).

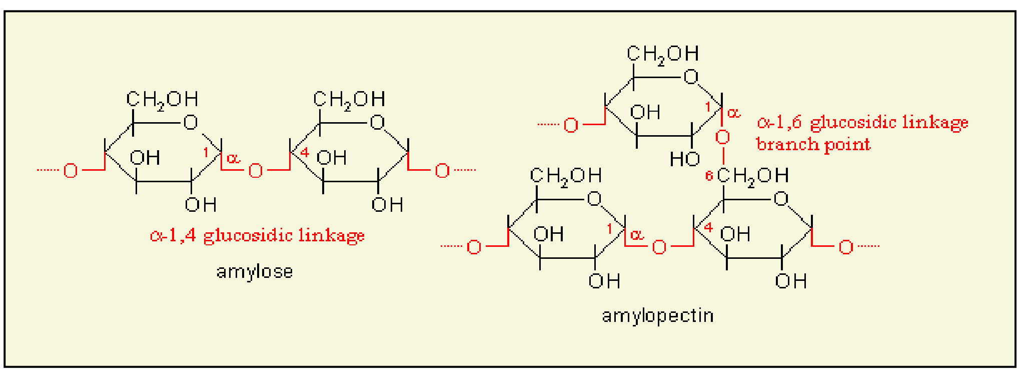

Figure 2.2: Section of a starch molecule (amylose and amylopectin)

Source: Keusch (2003)

Starch is one of the polysaccharides frequently used to develop edible films because it is a natural polymer that capable of forming a continuous matrix and it is a renewable and abundant resource (Bertuzzi et al., 2007). Moreover, starch also inexpensive polysaccharides, as well as biodegradable and nontoxic material. Flieger et al. (2003) draws a conclusion that converted starch to thermoplastic material offers an interesting alternative for synthetic polymers where long-term durability is not needed but rapid degradation is an advantage.

Edible films formulated with starch and glycerol had shown good mechanical properties when the tensile tests were performed (Fama et al., 2009). However, Flores et al. (2007) stated that the starch based films presented higher values of water vapor permeability and smaller elastic modulus than the ones of non-biodegradable packaging materials like polyethylene.

2.6 CHARACTERIZATION OF BIO COMPOSITE

There are several methods for characterization of the biodegradable films which are by using Universal Testing, water absorption test, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and soil burial degradation test and biodegradation test by using *Aspergillus niger*.

2.6.1 UNIVERSAL TESTING

A Universal Testing Machine also known as a materials testing machine and can be used to test the tensile and compressive properties of materials. This type of machines is called Universal Testing Machine because it can perform all kinds of tests like compression, bending, and tension to examine the material in all mechanical properties. This machine is used for a wide range of industries including materials testers for plastics, elastomers, textiles, adhesives, films, concrete, construction materials, biomaterials, medical devices, ceramic, bone, and metals.

From the previous studies, the tensile strength and tensile modulus are higher for the bacterial cellulose with starch composites compared to those of the unreinforced starch. The tensile strength of bacterial cellulose with starch composites is 2.03 to 2.34 times higher compared to the pure starch when fiber loading is 7.8 wt % to 22 wt %. The tensile modulus increases by 111.7% to 132.4% respectively at 7.8 wt % to 22 wt % fiber loading. Additionally, from research conducted by Liu et al. (2010) it shows that the tensile strength and Young's modulus for starch composite with additional of bamboo cellulosic crystals (BCC) will increase when the content of BCC increased from 0% to 8%. Both increased sharply from 2.5 to 12.8 MPa, and from 20.4 to 210.3 MPa respectively. According to Tongdeesoontorn et al. (2011), the increasing tensile strength attributable to the formation of intermolecular interaction between the hydroxyl group of starch and carboxyl group of cellulose. However, the elongation at break decreased when BCC content increased. This is because the high content of BC fillers might contribute to retarding the intermolecular interaction of the starch films (Wittaya, 2009).

2.6.2 WATER ABSORPTION

In the case of a product packaging whose deterioration is related to its moisture content, the barrier properties of the package relate to the water vapour which will be one of major importance in extending shelf life (Alves et al., 2006). In the BC and starch film, both starch and BC are hydrophilic that can cause the composites become high moisture absorption. However, the chemistry similarity may result in good interface adhesion between the two components. Dufresne et al. (2000) has been supported by state that the presence of cellulose micro fibrils within the starch material can decreases the water sensitivity. The present of cellulose can prevent the moisture absorbance by changing the poor interface adhesion with strong fibre-matrix to resist the diffusion of water molecule along the interface (Wan et al., 2009). Figure 2.3 shows the moisture absorption curves of BC/starch bio composites with different BC contents